

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

116

12/1/94

From Page No. \_\_\_\_\_

Purpose: To try new primers again with pM09  
to get rid of mispriming optimization of Mg

used: KlenTag buffer w/o Mg added Mg later, different conc  
5 µl

1 Unit Klg  
1 µl primer  
200 ng template  
200 µM dNTP

included:  
primer 1 alone  
2 "  
w/o primer  
w/o Mg as controls

mm	1	1.5	2	2.5
	5	7.5	10	12.5
	45	42.5	40	37.5
	50			
	1			
	5 µl / rx			

Cycling: 94° 3'  
30 ( 94° 30", 56° 30", 72° 3' )  
72° 60"  
4°

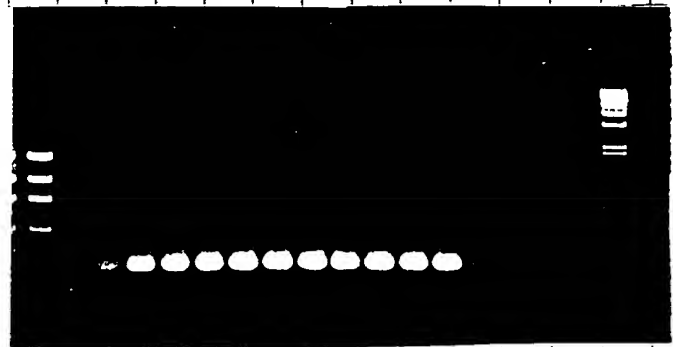
10 x buffer	60
dNTP	12
Template	2.4
enzyme	2.4
primer 1	6.0
2	6.0
H <sub>2</sub> O	45 / 1.2
	↓
45 µl + 5 µl	Mg

Did the same with new  
du primers

2720  
2729

10.7 + 10.6 primers 1 & 2  
1441.9 1420

w/o primers assembled separately



Witnessed & Understood by me,

*[Signature]*

Date

12/1/94

Investigated by

Recorded by

*[Signature]*

Date

12/2/94

T Page

g N .

Result: no product in both sets of reactions.

more primer dimer with new dU primers?

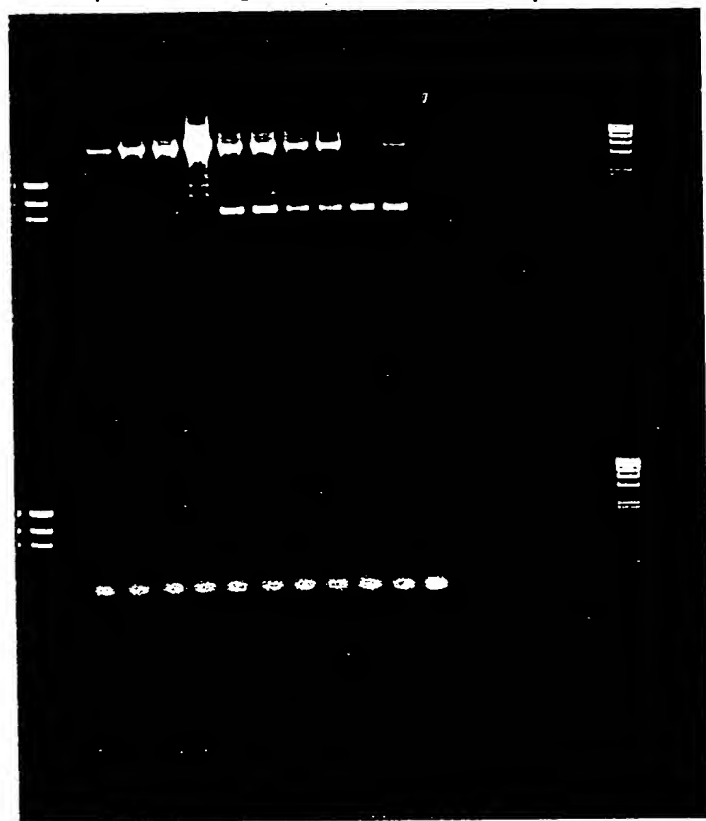
10 of Tag, 200 pg Template for 6.1 Kb - enzyme might not be enough. Did tag pg 102 - got products in KlenTag buffer but not of mispriming was observed.

Since it didn't work & no products were seen don't know about the effect of Mg.

repeated the expts again with DeepVent + Tag (0.01 + 1.0)

Result:

1d dU 1 1.5 2 2.5 3 mM Mg



New dU

Once again new dU primer didn't work even with this enzyme cocktail

- Less Mg = less mispriming the lower band almost disappears

- 3 mM has product than 1.5 - 2.5 mM

- Again with dU - new primer more primer dimer

\* \* Try with more enzyme Tag - 2U

To Page No. \_\_\_\_\_

sed & Understood by me,

Date

Invented by

Date

Recorded by

R. Liberman

12/2/94